Contributions to the embryology of the Ranunculaceæ.

WITH PLATES XVII–XX.

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(Concluded from page 248.)

Of the three species of Ranunculus examined, *R. abortivus* is most abundant in this vicinity thriving well in the dry soil of open woods and meadows, while the other two, *R. recurvatus* and *R. septentrionalis*, seem to prefer the moist soil of more dense and shady woods and along water courses. We shall direct our attention first to

Ranunculus abortivus.—The carpophyll may be said to consist of a short stalk or petiole and a broader portion, the lamina. The ovule arises as a small protuberance at the junction of petiole and lamina, but, of course, before any differentiation into these two parts is perceptible. The lamina soon broadens, the edges bending upward to form a crescent shaped cup or cavity into whose opening the rudimentary ovule projects. A longitudinal radial section of a carpel thus far developed, presents a picture closely resembling a partly closed hand with the thumb pointing downward toward the palm, the thumb representing the rudiment of the ovule (fig. 41*a*). At this stage of development the edges of the lamina have not quite organically united, a fact made clear by fig. 41*b* which is a transverse section cut in the plane *x*—*x* of fig. 41*a*.

The initial cell of the embryo-sac is hypodermal. The surrounding hypodermal cells by their behavior seem to be suggestive of a sort of archesporium (figs. 31, 32).

Before the first division it elongates a good deal, and sometimes becomes very broad (fig. 33). In the majority of cases observed only the lower of the two cells resulting from the first division divided again (fig. 38). Sometimes, however, both cells divide as in the preceding genera (fig. 39) though this is less frequent in the genus Ranunculus. In a number of instances the nucleus in the upper cell divided without being followed by the formation of a cell wall (fig. 35), and in others a longitudinal division of this cell took place. From fig. 34 it seems probable that two cells have begun to develop into embryo-sacs. Here only two cells were formed from

each initial cell, the upper being already partly disorganized. The development of the embryo-sac from the mother-cell is perfectly normal. In this species the egg-apparatus is larger at first than the antipodal cells (fig. 40) two of which only are shown in the figure. The latter increase in size, however, with the subsequent growth of the embryo-sac, but as far as observation went, they do not reach the enormous size that obtains in the genera previously mentioned.

Ranunculus recurvatus.—The most striking difference between R. abortivus and R. recurvatus from an histological point of view, is the larger meristematic cells of the latter. In this respect R. recurvatus resembles Delphinium and Caltha. The development of the embryo-sac from the initial cell requires but few additional remarks. Figs. 42 and 43 will make the process clear. Fig. 44 presents a phenomenon probably indicating the tendency of more than one cell to develop into an embryo-sac.

The antipodal cells are large, and increase considerably in size during the subsequent growth of the sac. In one case their position differed from their usual orientation (fig. 45). In one embryo-sac a cleft or cavity seemed to extend some distance downward into the chalaza beneath the antipodal cells, in which could be recognized only a slight trace of a protoplasmic substance (fig. 46). Here the large antipodal cells, rich in protoplasm, and with large nuclei, rest upon the disorganized remains of cells. Another exceptional phenomenon was observed in the presence of a small tracheary and a small tracheid element beneath the antipodal cells (fig. 60). In this figure are shown two antipodal cells of unequal size, one behind the other, with an endosperm nucleus on the right and left, and the above named elements below. As these two phenomena were observed in one case only, I am not inclined to attach any special significance to them at present.

R. septentrionalis.—This species resembles the preceding very closely, especially in regard to the size of its embryonic cells and the behavior of the initial cell, which gives rise sometimes to four cells (fig. 47). Fig. 48 illustrates what frequently takes place in the cells which immediately surround the embryo-sac mother cell and its disorganizing sister cells. The mother-cell in the figure is partly concealed by those on either side.

The mature embryo-sac is perfectly normal; the antipodal cells are large, and increase in volume with the subsequent growth of the embryo-sac.

Anemonella thalictroides.—In an early stage of the ovule of this species there was observed in several instances a number of hypodermal cells well marked off from the cells below, suggesting a primitive archesporium (figs. 49, 50). It is very probable that there is here but one initial cell, as only one was found to proceed in its development, and that the sharp definition of these cells is due to regularity in growth; yet the possibility is not excluded that there is present a tendency of more than one cell to become reproductive. As to the origin of the inner integument at *i—i*, fig. 49, there can be no question. In all ovules examined the number of cells produced by the initial cell was three (fig. 52). The nucellus is rather long and narrow impressing this character upon its cells.

The antipodal end of the gradually enlarging embryo-sac is more or less pointed, and the large antipodal cells adapt themselves to this space. Extending backward through the nucellus from the antipodal end of the newly formed embryo-sac is a strand of long narrow cells resembling the rudiment of a vascular bundle. This strand is gradually absorbed by the encroaching embryo-sac which makes its way wedge-like to the base of the nucellus. The nuclei of the antipodal cells are large and undergo division.

Thalictrum dioicum.—In this genus the development of the embryo-sac agrees closely with that of Anemonella. It is evident (fig. 54) that the great bulk of the nucellus owes its origin to the hypodermal layer of cells. This statement will apply generally to all the preceding genera.

As far as observation extended, the initial cell gives rise to three cells only, but in all probability four occasionally result from two successive divisions, as in the other genera.

The nucellus is less elongated than in Anemonella; the embryo-sac more oval in form and it reaches maturity sooner with reference to the corresponding growth of the integuments. The antipodal cells are large, but not so pronounced as in Anemonella.

Hepatica acutiloba.—The earlier stages in the development of the embryo-sac of Hepatica were not observed, as my material, collected about the middle of November, was too far

advanced for that purpose. The youngest stage observed showed three cells derived from the initial cell, the lower one becoming the mother cell.

The mature embryo-sac of Hepatica is especially interesting. Before fertilization both egg-apparatus and antipodal cells, as well as the cavity of the sac itself, become unusually large. At the upper ends of the synergidæ there appears a number of protoplasmic strands converging to a point and thus forming a star-shaped cap (fig. 55). The egg-cell is broader at its upper end and rather deeply inserted. All three cells of the egg-apparatus are surrounded by very delicate membranes. The antipodal cells increase greatly in size during the subsequent growth of the embryo-sac, and at the time of fertilization each cell may contain as many as ten or a dozen nuclei (fig. 56), due to fragmentation. The process of fragmentation may be readily observed here (fig. 57). Each nucleus contains two or more nuclei situated in spherical or oval vacuoles.

Soon after fertilization, or when the embryo has reached the stage of development shown in fig. 58, the antipodal cells bear evidence of dissolution, the fusion of all the nuclei into a common mass being the preparatory step.

On account of the large nuclei, the cells of the developing embryo and endosperm of Hepatica afford favorable objects for the study of karyokinesis.

I may add here that by staining on the slide with aniline safranine and picric nigrosine I was able to see the centrospheres or what seemed to be centrospheres (fig. 69). The centrosomes appeared as extremely small dark points surrounded by a colorless court, and the radiating kinoplasmic zone was also darker but difficult to see.

In some instances I have observed what were taken to be centrospheres at the poles of nuclear spindles (fig. 4, upper end of spindle) in preparations that were not subjected to the special process said to be necessary to demonstrate these structures.

Summary.—This work has been interesting especially as a study in variation. That which first of all attracts our attention is found in the presence of more than one initial cell and their development into normal embryo-sacs. In the genera under consideration the number of initial cells reaches its culmination in Caltha where five or more may be present

in the ovule, suggesting a sporangium which indeed it is, for the ovule certainly stands for a macrosporangium with its integumentary covering or indusium. Although the presence of more than one embryo-sac in an ovule has been observed by several investigators in widely separated families, yet further research will undoubtedly show the phenomenon to be still more prevalent and widely distributed. Just how far this fact will throw light upon the origin of the angiosperms remains yet to be seen, but it certainly contributes materially to the phylogeny of the seed plants.

The production of a series of four cells by two successive divisions of the initial cell of the embryo-sac, occurs more frequently in this family than had hitherto been supposed; and it seems probable that a more extended search in those genera in which this phenomenon was not observed by myself would reveal its occasional presence.

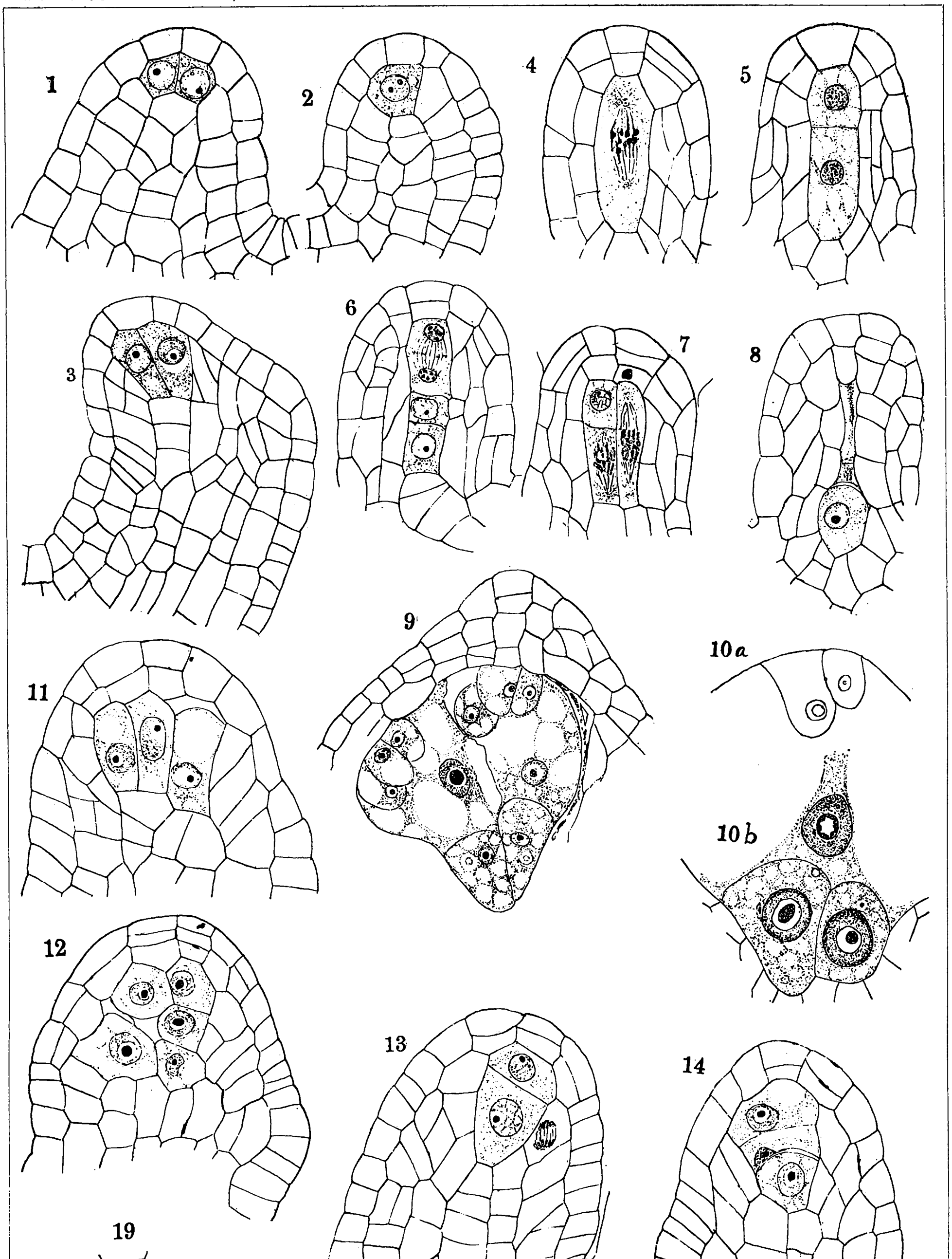
In connection with the foregoing facts several questions naturally arise upon which it is not my purpose to speculate at present. What is the significance of the fact that in the same species the initial cell gives rise sometimes to only three cells and sometimes to four? Is the behavior of the initial cell due to inherent tendencies alone, or is it determined partly by external forces? Which is the more primitive condition, that represented by the series of three or of four cells?

If the small cell cut off occasionally from the initial cell in Aquilegia be regarded as a tapetum, we certainly have here an isolated phenomenon occurring where it would not be generally expected. Guignard[10] has observed the presence of a tapetum in certain genera of a family while in other genera of the same family a tapetum was absent; but no other instance is now known to me in which a tapetum was only of occasional occurrence in the same species.

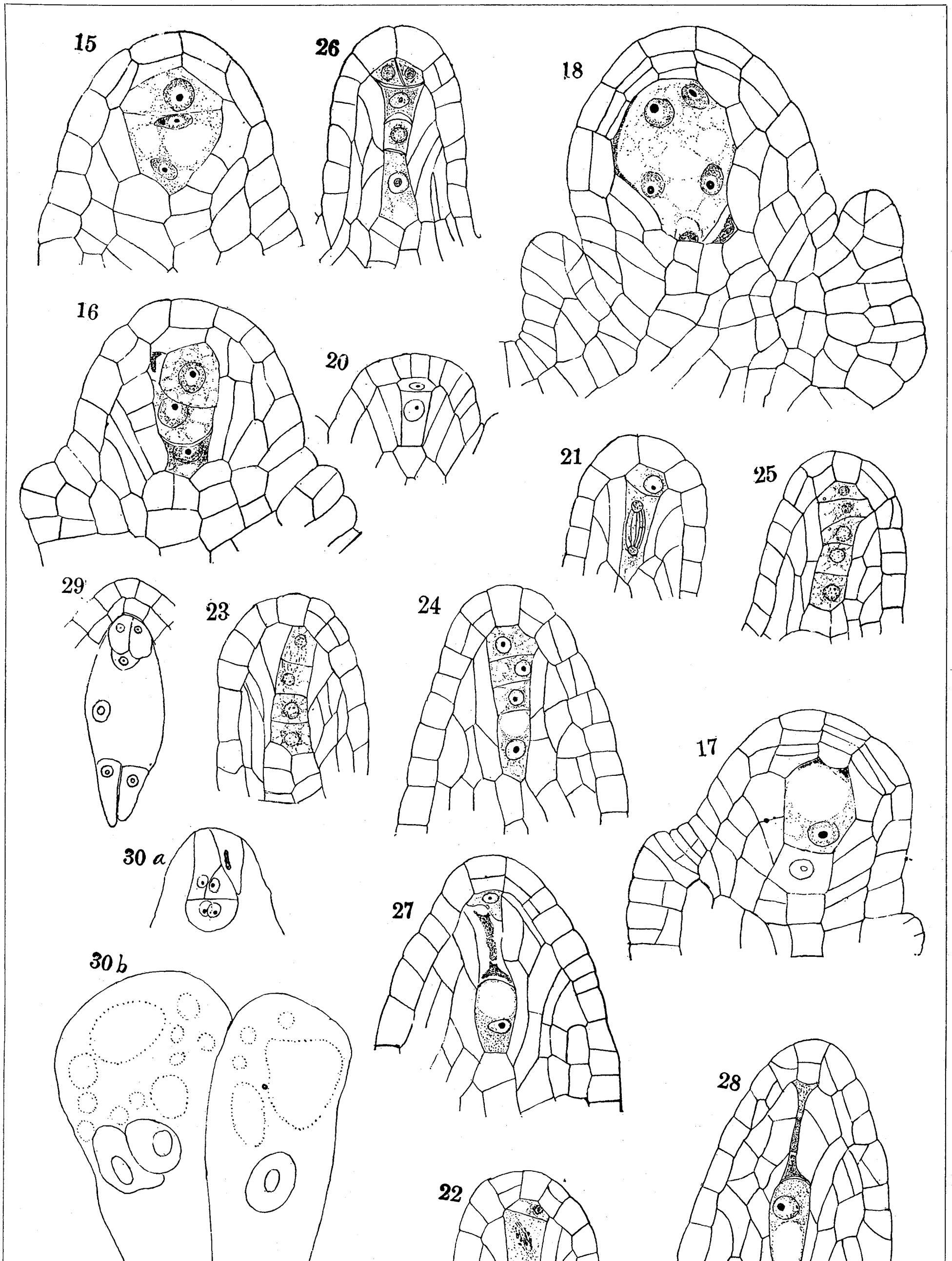
The rule so general in its application among angiosperms, that the lower cell of the series derived from the initial cell develops directly into the embryo-sac, seems to have a probable exception in Caltha (fig. 16). Guignard speaks also of a probable exception in *Acacia albida*, and in the same place refers to a case reported by Mellink in one of the monocotyledons (Agraphis), together with the analogous case described by Strasburger for *Rosa livida*.[11]

[10] Embryogénie des Legumineuses. Ann. des Sc. Nat. Bot. VI. **12**: ——. ——.

[11] l. c., p. 137.



MOTTIER on RANUNCULACEÆ.



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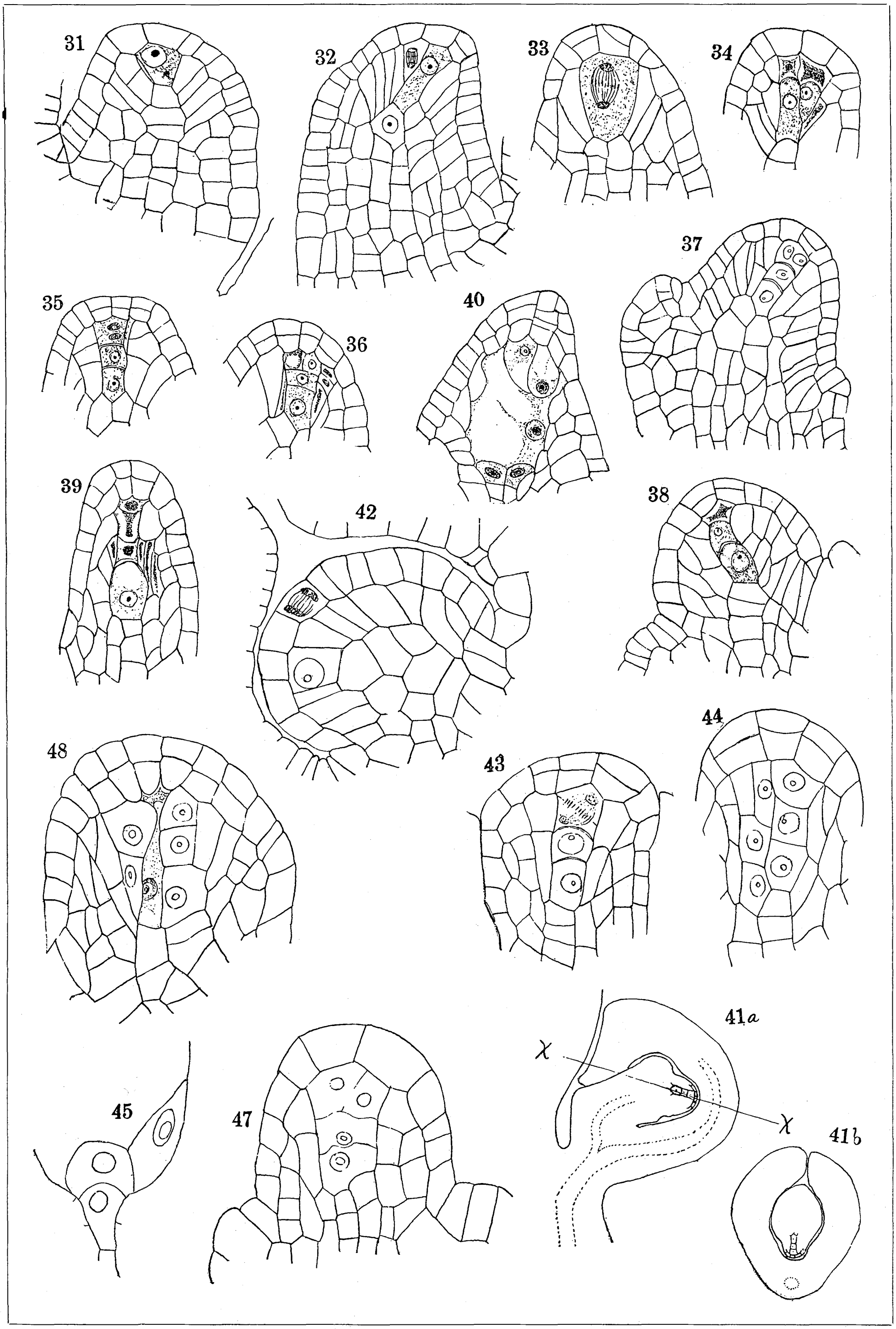

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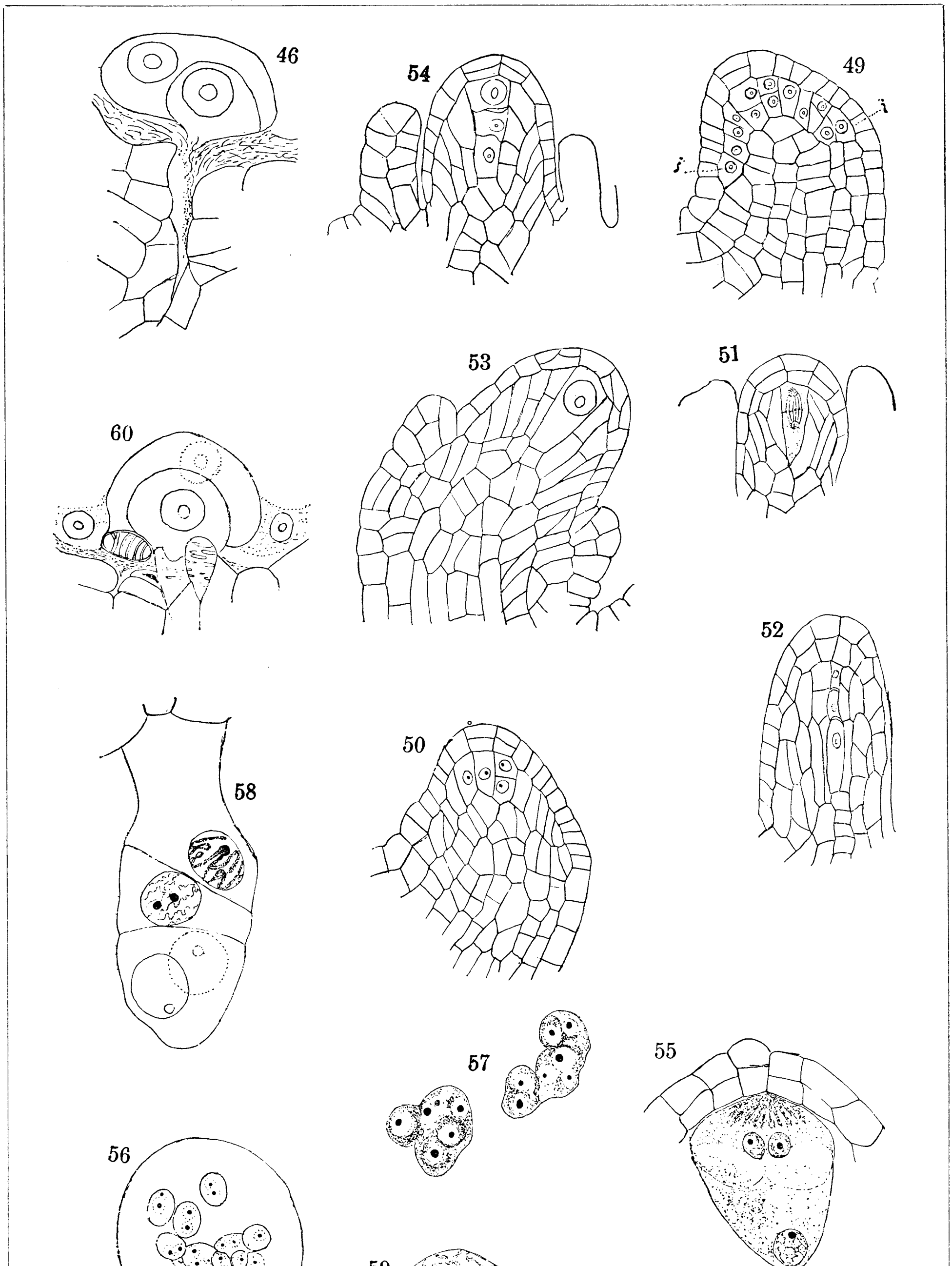

MOTTIER on RANUNCULACEÆ.

In regard to the large cells of the ovule in such species as Caltha palustris, Ranunculus septentrionalis and Delphinium tricorne and the comparatively small cells of Aquilegia, it seems that we have a phenomenon which may be correlated with the habitat of the plant. In the marsh dwelling Caltha we find the largest cells, followed in order by Ranunculus septentrionalis, R. recurvatus which thrive better in moist soil; then come Delphinium, Hepatica and R. abortivus which flourish in a drier soil. On the other hand Anemonella, Thalictrum, and especially Aquilegia, whose cells are very small, are particularly dry soil plants. This is not given as a theory or explanation, but merely as a suggestion, for I do not know how far the facts in different genera and species of other families contribute to the support of such a view or whether they contradict it.

Besides the Ranunculaceæ, unusually large antipodal cells have been observed in *Delphinium villosum*,[12] *Ornithogalum nutans*, *Gladiolus communis* and *Crocus*. I have also observed large antipodal cells in *Jeffersonia diphylla*.

As to the large antipodal cells, which with their numerous nuclei become so rich in protoplasm, it may be said in harmony with known facts, that they have passed from a formative into a nutritive condition which we find paralleled in the internodal cells of Chara and Nitella.[13]

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Explanation of Plates XVII–XX.

Figs. 1–10, Delphinium.

Figs. 1 and 2. Median longitudinal sections of very young ovule. × 335. In fig. 1 two initial cells can be seen; in fig. 2, only one, which is larger than the other hypodermal cells.—Fig. 3. Similar section of an older ovule with two large initial cells. × 335. The anatropy of the ovule is more pronounced.

Figs. 4–8. Median longitudinal sections of the nucellus. Fig. 4. The nucleus of the large initial cell of the embryo-sac is in the spindle stage of division; at the upper pole are centrospheres. × 335.—Fig. 5. The initial cell has divided transversely, the lower cell being larger. × 335.—Fig. 6. The lower cell has already divided; the upper is in process of division. × 335.—Fig. 7. Two initial cells in process of development. In the cell on the right, the nucleus is dividing; the one on the left shows the lower cell in process of division. × 335.—Fig. 8. The lower cell of the series, the embryo-sac-mother-cell, has en-

[12] Strasburger, Ueber Befruchtung und Zelltheilung, 38, 41. 1878.
[13] Strasburger, Histologische Beiträge, 5: 99, 100, etc. 1893.

larged considerably; the three cells of the series above are disorganized. × 335.

Fig. 9. Two mature embryo-sacs lying side by side in the same ovule. × 260.—Fig. 10, *a* and *b*. The egg-apparatus and antipodal cells respectively drawn from the same embryo-sac. Only one synergida is shown, the other lies beneath. Above the antipodal cells, of which two are drawn, lies the large endosperm nucleus. × 335.

Figs. 11–19, Caltha.

Figs. 11 to 18. Median longitudinal sections of young ovules.

Fig. 11. Three large initial cells stand side by side in the plane of the section. Some of the epidermal cells above them have divided by periclinal walls. × 335.—Fig. 12. Probably a group of four or five initial cells; a space is seen between the two on the left. × 335.—Fig. 13. The initial cell has divided, and the nucleus of the lower cell is in the skein stage preparatory to division. × 335.—Fig. 14. Three cells have been derived from the initial cell. The mother-cell already slightly enlarged is separated above by a swollen wall. ×335.—Fig. 15. Two cells have been formed from the initial cell; the two nuclei in the lower are separated by two large vacuoles. × 335.—Fig. 16. Of the three cells derived from the initial cell the two upper are larger; the lower cell seems partly crowded out; the inner integument has just appeared. × 335.—Fig. 17. The mother-cell has apparently displaced the two cells above it. The epidermis of the nucellus has formed a layer three or four cells thick. × 335.—Fig. 18. Embryo-sac with two nuclei in each end. × 335.

Fig. 19. In the upper cell formed by the first division of the initial cell two nuclei are shown in outline. × 335.

Figs. 20–30. Aquilegia.

Figs. 20–28. Median logitudinal sections of the nucellus.

Fig. 20. A small cell has been cut off from the initial cell. ×465.—Fig. 21. Similar to Fig. 20; the nucleus of the larger cell is in division. × 465.—Fig. 22. One step beyond fig. 21; in the two cells below the small tapetal (?) cell, the nuclei are in division. ×465.—Fig. 23. Only the usual four cells have been produced by the initial cell. ×465.—Fig. 24. Same as fig. 23, the mother cell having enlarged. × 465.—Fig. 25. The initial cell has given rise to five cells. × 465.—Fig. 26. The terminal cell of the series has divided almost at right angles to the usual plane of division. × 465.—Fig. 27. The enlarging mother cell with the remains of the sister cells above; a large vacuole occupies the upper half of the mother-cell. × 465.—Fig. 28. Similar to fig. 27, but the nucellus is narrower; the vacuole occupies the lower half of the cell. × 465.

Fig. 29. An embryo-sac shortly after maturity. The nucellar cap is only two cells thick. × 335.

Fig. 30, *a* and *b*. A young embryo with the remains of one of the synergidæ and two enormous antipodal cells drawn from the same embryo-sac. Two nuclei are present in the cell on the left; vacuoles are indicated by dotted lines. × 260.

Figs. 31–41. Ranunculus abortivus.

Figs. 31–38. Median longitudinal sections of the ovule.

Fig. 31. The initial cell is distinguishable by its larger size. × 335.—Fig. 32. The initial has increased greatly in length; the cell of the axial row of the nucellus, just below it failed to divide, and as a result is larger than its neighbors. × 335.—Fig. 33. The nucleus of the unusually broad initial cell is in division. × 335.—Fig. 34. Two initial cells that have each given rise to two cells which are apparently mother-cells. One mother-cell is partly hidden by the other. × 335.—Fig. 35. Three cells have resulted from the initial cell, the nucleus in the upper one has divided. × 335.—Figs. 36 and 37. Similar to fig. 35. In fig. 36, the uppermost cell has divided longitudinally. In 37, the two nuclei in this cell lie side by side in a vertical plane. × 335.—Fig. 38. The mother-cell has begun to encroach upon the cells above. × 335.

Fig. 39. Four cells have evidently been produced from the initial cell. The disorganizing cells above the large mother cell have greatly swollen walls. × 335.

Fig. 40. Mature embryo-sac, together with the surrounding tissue of the nucellus. Only two antipodals and two cells of the egg-apparatus are drawn. × 260.

Fig. 41, *a* and *b*. *a*, a longitudinal radial section through an immature carpophyll; *b*, a transverse section made in the line *x*—*x*. × 85.

Figs. 42–46, 60, Ranunculus recurvatus.

Fig. 42. Median radial longitudinal section of a rudimentary ovule. The nucleus of the initial cell is drawn in outline. × 335.

Figs. 43 and 44. Median longitudinal sections of the nucellus. In fig. 43, four cells would have resulted from the initial cell, the division of the upper cell is almost complete, when the wall would have been oblique. In fig. 44, three cells have resulted from the initial cell (on the right), and if two initials were present here, four cells (on the left) would have been the descendants of the same. × 335.

Fig. 45. Three antipodal cells, with an unusual orientation.

Fig. 46. A vertical section through a portion of the chalaza. Two antipodal cells rest upon fragments of disorganized cells, and below them a narrow cavity extends a short distance downward into the chalaza. This figure was taken from an embryo-sac that had increased greatly in size after having been laid down. × 335.

Fig. 60. Two antipodal cells, below which are two small tracheary elements: on the right and left may be seen a nucleus of the endosperm. × 335.

Figs. 47, 48, Ranunculus septentrionalis.

Fig. 47. Longitudinal section of nucellus. Four cells have been formed from the initial cell; the upper one has divided obliquely. × 335.

Fig. 48. The enlarging mother cell is partly hidden by the lateral, turgid cells. × 335.

Figs. 49–52. Anemonella.

Figs. 49, 50. Longitudinal section of the ovular rudiment. Several hypodermal cells with nuclei indicated are very sharply defined from the cells beneath. The origin of the inner integument is shown at *i, i*. In Fig. 50 the hypodermal cells are equally well defined; the initial cell is probably the middle one of those in which the nuclei are outlined. × 335.

Figs. 51, 52. Longitudinal sections of the nucellus.

Fig. 51. The nucleus of the initial cell is in process of division. × 335.—Fig. 52. Three cells have been derived from the initial cell; the mother-cell has somewhat enlarged. The nucellus is long and narrow. × 335.

Figs. 53–54. Thalictrum.

Fig. 53. Longitudinal section of young ovule. The initial cell is very large, reaching almost the entire length of the nucellus. This figure plainly shows that the bulk of the nucellus, aside from the epidermis, owes its origin to the hypodermal layer of cells. × 335.

Fig. 54. Three cells owe their origin to the initial cell. × 335.

Figs. 55–59. Hepatica.

Fig. 55. Egg-apparatus with the nucellar cap above. At the upper ends of the synergidæ is a star-shaped protoplasmic mass. × 260.

Fig. 56. An antipodal cell with eleven nuclei due to fragmentation. × 260.

Fig. 57. Two nuclei from an antipodal cell in process of fragmentation. × 335.

Fig. 58. A four-celled embryo. The wall separating the lower end cells lies almost in the plane of the paper. The nucleus indicated by dotted line could be seen by focusing down. × 335.

Fig. 59. Nucleus with two centrospheres at the right. × 675.